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Abstract

Presented herein is the construction of pipettor tips (termed MSIA-Tips) containing porous solid supports that are constructed, covalently derivatized with affinity ligand, and used to extract specific proteins and their variants from various biological fluids by repeatedly flowing the fluids through the MSIA-Tips. A second protein species (a mass-shifted variant of the targeted protein doped into the samples at a constant concentration) is co-extracted with the endogenous protein and variants and is used as a quantitative internal reference standard (IRS). Nonspecifically bound compounds are rinsed from the MSIA-Tip using a series of buffer and water rinses, after which the wild type protein, protein variants and the IRS are eluted from the MSIA-Tips directly onto a target in preparation for analysis such as MALDI-TOF. Mass spectrometry of the eluted sample then follows with the retained proteins identified via accurate molecular mass determination. Protein and variant levels are determined via a quantitative method in which the protein/variant signals are normalized to the signal of the IRS and the values compared to a working curve constructed from samples containing known concentrations of the protein or variants.